韓國臨床獸醫學會誌 第8卷 第1號
Korean J. Ved. Clin. Med.
Vol. 8, No. 1, June, 1991

Diagnosis and Chemotherapy of Mycotic Mastitis

Mahendra Pal and **Chang-Woo Lee***

Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, Gujarat Agricultural University, Anand-388001, India

Department of Clinical Pathology, College of Veterinary Medicine, Seoul National University, Republic of Korea*

Summary

Opportunistic mycotic infection of mammary gland due to *Candida tropicalis* is described in a 7-year-old Holstein Friesian cow with a history of prolonged use of antibiotics and corticosteroids. *C. tropicalis* was repeatedly isolated form the mastitic milk on simplified sunflower seed medium at 30℃. The pathogen was directly detected in the infected milk by 'PHOL' technique. Microscopic examination of Gram stained smears of milk sediment revealed numerous yeast cells and hyphae morphologically consistent with *Candida* spp. *In vitro* drug sensitivity test indicated that *C. tropicalis* was sensitive to nystatin. Chemotherapy with intramammary infusion of nystatin was successful as evidenced by clinical and mycological observations. It emphasizes the growing significance of *Candida* spp. and other opportunistic fungal pathogens in Veterinary medicine.

Introduction

Mastitis, a disease of diverse etiologies, is important from economic as well as public health point of view. Among the infectious causes, bacteria are implicated as the chief etiologic agents of mamary gland infection in dairy animals.[3,4] However, sporadic reports on the association of fungi with mastitis have also appeared in the literature.[9,10,17] The purpose of this communication is to put on record a case of mastitis in a cow due to *C. tropicalis* and its successful treatment with nystatin. In addition, it also describes the efficacy of 'PHOL' stain and sunflower seed culture medium for the study of *Candida* organisms.

Materials and Methods

A seven-year-old Holstein Friesian cow was presented to the Veterinary Hospital with a history of inflammation of mammary gland and reduced milk yield. The animal was recently parturited and delivered a normal female calf. She developed swelling of the hind quarter after 20 days of calving. The udder was thoroughly washed with potassium permanganate(1 : 500) solution and then dried with sterile towel. The mid-stream milk samples(8~10㎖) collected aseptically in sterile McCartney glass stoppered bottle after cleaning the teats with single use alcohol(70%) swab, were submitted to the laboratory for culture and durg sensitivity. The samples were collected on three different occasions after an interval of 24 hrs and processed immediately without any further delay. A heavy loopful of the specimens from the infected teats was streaked onto the plates and slants of nutrient agar and

simplified sunflower (*Helianthus annus*) seed medium[14] (sunflower seed 45g, agar 20g, chloramphenicol 100mg, distilled water 1000mℓ), Both the media were incubated at 30℃ and examined daily for microbial growth. About 5mℓ of the milk was centrifuged at 3000 r.p.m. for 5 minutes and a drop of the sediment was treated with 1 drop of 'PHOL' stain[15] and examined directly under microscope for fungal elements, if any. Smears were also prepared on the clean glass slides from the centrifuged deposit of the milk and screened after staining with Gram's technique. The detailed identification of the organism was made according to Carter.[4] The drug sensitivity of the isolates against nystatin(100μg) was conducted by a standard disk diffusion method.[2] Based on the sensitivity report, the animal was treated with nystatin(500,000 I.U. dissolved in 10mℓ of sterile distilled water). The drug was infused into the infected teat daily for 4 days. The milk specimen obtained after 14 days of the last medication, was cultured on simplified sunflower seed medium to evaluate the efficacy of th chemotherapeutic agent.

Results

Physical examination revealed that left rear quarter of the cow was hard, swollen and milk contained white clots. The milk flow from the affected teat was reduced. However, there was no systemic signs such as fever, anorexia, depression, inappetence and ataxia. The animal was treated with tetracyclines and chloramphenicol for a week but there was no clinical response.

C. tropicalis was repeatedly isolated from the mastitic milk on simplified sunflower seed medium. Many small, round, smooth, whitish-cream coloured colonies of the yeast were observed on simplified sunflower seed medium after 72 hrs of incubation at 30℃(Fig. 1). No other pathogen was recovered from any of the 3 samples of mastitic milk when cultured on three different occasions after a gap of 24 hrs. Microscopic examination of milk sediment in 'PHOL' stain revealed many oval, thin walled 2-4μ in diameter yeast with hyphae resembling *Candida* spp.(Fig. 2).

Similar fungal bodies were demonstrated in the Gram stained smear of the centrifuged milk deposit-(Fig. 3). On the basis of growth, morphological and biochemical findings, the isolates were confirmed as *C. tropicalis*.

In vitro testing of the drug by single disk diffusion technique indicated that all the three isolates of *C. tropicalis* were found to be sensitive to nystatin(100μg).

The infusion of nystatin(500,000 I.U.) into the affected quarter was effective in treating mycotic mastitis due to *C. tropicalis*. The milk specimen after treatment failed to reveal the growth of the fungus on the culture medium. The drug showed good response; and no relapse was observed when the animal was followed after 2 months.

Discussion

Among the fungi, *Candida* is the most commonly encountered yeast from the secretion of mammary gland[7]. Various species of the genus *Candida* such as *C. albicans, C. tropicalis, C. krusei C. parapsilosis, C. guilliermondii, C. pseudotuberculosis, C. rugosa* are isolated from the mastitic milk of dairy animals.[6~8,11,18,19] The repeated isolation of *C. tropicalis* from the infected milk, its direct demonstration in the milk sediment by 'PHOL' and Gram's techniques, clinical history of the case and response to nystatin undoubtedly establish the etiologic role of this opportunistic yeast with bovine mastitis.

The literature review indicate that *C. tropicalis* is associated with mycotic mastitis in cows and buffaloes.[1,5,8,10,19] Some of these reports did not mention the results of the direct microscopy. As *Candida* spp. commonly occur as commensal in the gastrointestinal tract and on the skin of healthy animals,[20~22] the mere recovery of the yeast from such clinical specimen can not conclusively prove the pathogenic significance. Therefore, it is highly imperative to demonstrate the presence of fungal elements in clinical specimen or to attempt repeated isolation of the organism.

The clinical signs of mastitis caused by fungi are not characteristic to warrant a diagnosis of mycotic mastitis. The animal with bacterial infections of the udder also exhibits the same symptoms and therefore,

presents diagnostic and chemotherapeutic problems. Hence, cultural isolation of the specimen must be carried out in all cases of mastitis to confirm the causative agent. Most of cows with *Candida* infections of udder show good prognosis, as animal starts producing normal milk.[6] The results of the present investigation suggest that nystatin can be safely recommended for the chemotherapy of *Candida* mastitis. The similar findings have been recorded by earlier worker.[12]

It has been mentioned that diluent used for the preparation of intramammary infusion should be sterilized as in majority of cases infection is introduced through the contaminated antibiotics preparation.[9] Hence, proper sterilization of dairy utensils, sanitary measures during intramammary infusion of drugs, personal hygiene of the milk atteddants will be of prime importance in the control of mycotic mastitis.

Good growth of *C. tropicalis* on simplified sunflower seed agar from the mastitic milk suggests the wider application of this simple medium by the diagnostic laboratories for the isolation of *Candida* spp. and other yeasts from a wide variety of clinical specimens. The medium originally formulated by Pal and Mehrotra[16] and later simplified by Pal and Baxter[14] has proved as an excellant selective medium for the rapid identification of *Cryptococcus neoformans* from clinical and environmental materials.[13]

Acknowledgements

Thanks are due to the veterinarian and other staff of the veterinary hospital for the submission of milk specimens and detailed clinical history of the patient.

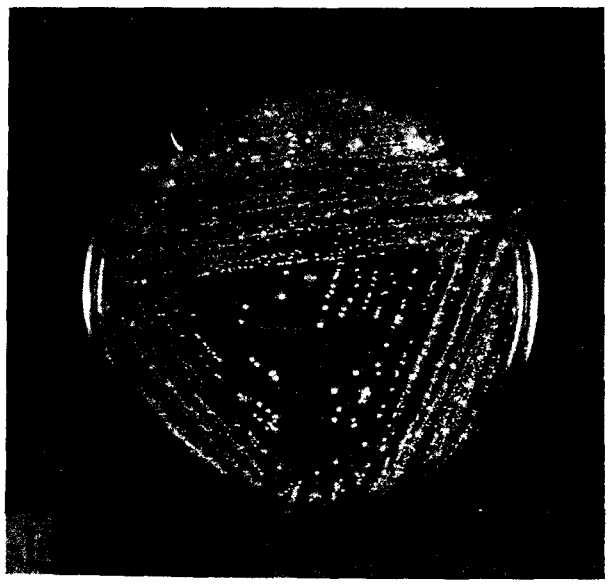
Present address for correspondence and reprint :
DR. M. Pal, M.V.P.H.; Ph. D., D.Sc.
Department of Veterinary Public Health
College of Veterinary Science and Animal Husbandry
Gujarat Agricultural University Campus
Anand-388001, Gujarat, India

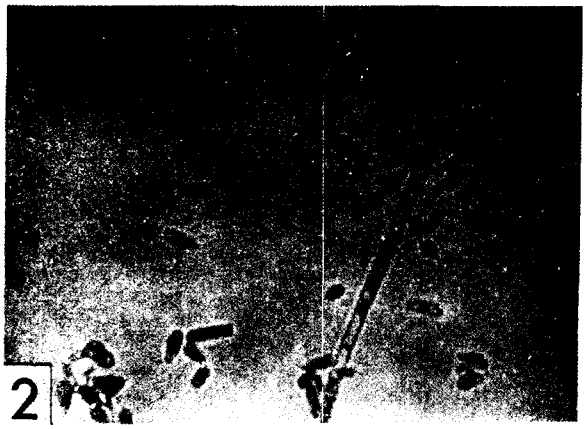
Legends for Figures

Fig. 1. Many small, circular white cream coloured colonies of *Candida tropicalis* on simplified sunflower seed medium, isolated from mastitic milk of a 7-year-old Holstein Friesian cow after 72 hours of incubation at 30℃.

Fig. 2. Microphotograph of milk sediment preparation showing oval yeast cells with hyphae of *Candida* spp. PHOL stain.×400.

Fig. 3. Oval yeasts and hyphae of *Candida* spp. evident in centrifuged deposit of mastitic milk. Gram stain.×400.





2

3

References

1. Ainsworth, G.C. and Austwick, P.K.C. : A survey of animal mycosis in Britain : Mycological aspects-Trans. Brit. Mycol. Soc., (1955) 38 : 369~386.
2. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. : Antibiotic susceptibility testing by a single disk diffusion method. Am. J. Clin. Pathol., (1966) 45 : 493~496.
3. Blood, D.C. and Radostits, O.M. : Veterinary Medicine. English Language Book Society, Bailliere, Tindall, London, (1989).
4. Carter, G.R. : Diagnostic Procedures in Veterinary Bacteriology and Mycology. 4th ed. Charles C. Thomas, Illinois, U.S.A., (1984).
5. Ceccarelli, A. : Qualche ricerea su di un Focaolaio dimastite de *Candida tropicalis* nel bovino. Zooprofilassi. (1961) 16 : 95~102.
6. Farnsworth, R.J. : Significance of fungal mastitis. J. Am. Vet. Med. Asso., (1977) 170 : 1173~1174.
7. Farnsworth, R.J. and Sorenson, D.K. : Prevalence and species distribution of yeast in mammary glands of dairy cows in Minnesota. Can. J. Comp. Med., (1972) 36 : 329~332.
8. Jand, S.K. and Dhillon, S.S. : Mastitis caused by fungi. Ind. Vet. J., (1975) 52 : 125~128.
9. Loftsgard, G. and Lindquist, F. : Bovine mycotic mastitis. Acta. Vet. Scand., (1960) 1 : 201~220.
10. Loken, K.L., Thompson, E.S., Hoyt, H.H. and Ball, R.A. : Infection of the bovine udder with *Candida tropicalis*. J. Am. Vet. Med. Ass., (1959) 134 : 402~403.
11. Monga, D.P. and Kalra, D.S. : Prevalence of mycotic mastitis among animals in Hariana. Ind. J. Anim. Sci., (1971) 41 : 813~816.
12. Pal, M. : Studies on fungi of cattle and buffaloes. D.Sc.(Doctor of Science) Thesis, Utkal University, Bhubane shwar, India (1987).
13. Pal, M. : Studies on animal and human cryptococcosis : A global saprozoonosis. J.S.P.S. Research Project Report. Department of Veterinary Internal Medicine, University of Tokyo, Japan (1990).
14. Pal, M. and Baxter, M. : Isolation of *Cryptococcus neoformans* from the environment using a simplified sunflower seed medium. Proc. New Zealand Microbiol. Soc., (1985) 29 : 155~158.
15. Pal, M., Hasegawa, A., Ono, K. and Lee, C.W. : A new staining solution for the morphological studies of fungi and *Prototheca*. Jpn. J. Vet. Sci., (1990) 52 : 527~531.
16. Pal, M. and Mehrotra, B.S. : Studies on the efficacy of sunflower seed agar medium for the isolation and identification of *Cryptococcus neoformans*. Arogya-J. Hlth. Sci., (1982) 8 : 74~79.
17. Prasad, L.B.M. and Prasad, S. : Bovine mastitis caused by a yeast in India. Vet. Rec., (1966) 79 : 809~810.
18. Richard, J.L., McDonald, J.S., Fichtner, R.E. and Aderson, A.J. : Identification of yeasts from infected bovine mammary glands and their experimental infectivity in cattle. Am. J. Vet. Res., (1980) 41 : 1991~1994.
19. Singh, M.P. and Singh, C.M. : Fungi isolated from clinical cases of bovine mastitis in India. Ind. J. Anim. Hlth., (1968) 7 : 259~263.
20. Smith, J.M.B. : Opportunistic Mycoses of Man and other Animals. C.A.B. International Mycological Institute, Wallingford, U.K., (1989).
21. Uden, N. van : The occurrence of *Candida* and other yeasts in the intestinal tracts of animals. Ann. N.Y. Acad. Sci., (1960) 89 : 59~68.
22. Williams-Smith, H. : Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Path. Bact., (1965) 89 : 95~122.

소의 진균성유방염의 진단과 화학요법 1예

엠팔 · 이창우*

인도 구자라트 농과대학 수의공중보건학교실 · 대한민국 서울대학교 수의과대학*

요 약

항생물질과 부신피질홀몬을 장기간 투여한 7세의 홀스타인종 암소에 있어서 *Candida tropicalis*에 의한 유방의 기회성진균감염을 보고하였다. *C. tropicalis*는 섭씨 30도의 단순한 해바라기씨 배지에서 반복하여 분리되었다. 이 병원체는 PHOL 기법에 의해 감염된 우유에서 직접 검출되었다. 우유침전물을 그람염색하여 현미경하에서 검사한 결과 *Candida* spp. 와 일치하는 다수의 효모세포와 균사가 관찰되었다. 실험실내 약물감수성을 조사한 결과 nystatin에 감수성을 나타내었다. 임상적 및 진균학적 검사결과 nystatin에 의한 유방내 화학요법은 성공적이었다. 수의학에 있어서 *C. tropicalis*와 기타 기회성진균의 점증하는 중요성이 강조되었다.